Cloning of the *Drosophila* ribosomal protein S3: another multifunctional ribosomal protein with AP endonuclease DNA repair activity

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A cDNA which encodes the *Drosophila* ribosomal protein S3 has been isolated from a 5.5 to 7.5 day pupal lambda gt10 library using the rat S3 cDNA as a probe. The *Drosophila* clone includes a single open reading frame of 738 nucleotides which predicts a protein of 246 amino acids with a molecular weight of 27,470 D. The initiation codon occurs in the context of CAAATGG which conforms to an optimal translation start consensus sequence. In addition, two potential polyadenylation sequences AAUACA and AACAAA, exist at the 3' end of the cDNA clone. The polypeptide deduced from the *Drosophila* sequence is 80% identical to the human (1) and rat (2) ribosomal protein S3. Most of the differences between the *Drosophila* and the human and rat amino acid sequences occur at the amino and carboxy termini (Figure 1). The *Drosophila* protein contains a nuclear localization signal (NLS), KKRK, and is proline (P) rich at the carboxy terminus, having 12 proline residues out of the terminal 55 amino acids. Moreover, the *Drosophila* protein contains well defined calcium/calmodulin-dependent protein kinase (R-X-X-S/T) and cAMP-dependent protein kinase (R-X-S/T) phosphorylation site, which are also features of the human and rat proteins (3). These phosphorylation sequences may play a role in regulating the activity as well as the localization of the protein. Furthermore, the *Drosophila* protein also contains a potential Asn (N) glycosylation site which is absent from the human and rat polypeptides. In studies not presented here, antibodies generated to *Drosophila* S3 fusion proteins have detected a single protein species which is associated with the nuclear matrix and chromatin, as well as ribosomes.

Amino acid residues from position 107 to 134 of *Drosophila* protein S3 exhibit 52% similarity and 37% identity with a region of the RNA polymerase β subunit, while residues 179 to 194 are 50% similar and 38% identical to a portion of the *Drosophila* topoisomerase II gene product (data not shown). Finally, a region of the *Drosophila* S3 protein between amino acid residues 54 and 94 has 50% similarity to a portion of the recently cloned *nuc2* gene from yeast (D.Burbee, San Diego, personal communication).

Biochemical analysis using overexpressed S3 protein in *E.coli* has revealed that *Drosophila* S3 is an AP endonuclease that cleaves DNA at AP sites by β-elimination (type I) reaction (data not shown). This finding is intriguing in light of the multifunctional activities of ribosomal protein S6 which is a tumor suppressor gene in *Drosophila* involved in the hematopoietic system (4) and our previous finding that *Drosophila* ribosomal protein AP3/PO is also an AP endonuclease acting through a hydrolytic (type II) cleavage mechanism (5, 6). These latter results and the mechanism that S3 uses to associate either with the ribosome or in the nucleus are under investigation.

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REFERENCES

1. Zhang,X.T., *et al.* (1990) *Nucleic Acids Res.* **18**, 6689.
2. Chan,Y.-L. *et al.* (1990) *Arch. Biochem. Biophys.* **283**, 546–550.
3. Kennelly,P.J. *et al.* (1991) *J. Biol. Chem.* **266**, 15555–15558.
4. Watson,K.L. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11302–11306.
5. Kelley,M.R. *et al.* (1980) *Mol. Cell. Biol.* **9**, 965–973.
6. Grabowski,D.T. *et al.* (1991) *Nucleic Acids Res.* **19**, 4297.

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Human   1 MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVTPTRTEIII   50
Rat         N
Dros  1 MNANLP      S                              S

Human  51 LATRTQNVLGEKGRRIRELTAVVQKRFGFPEGSVELYAEKVATRGLCAIA  100
Rat
Dros     M  K  Q            M    N ET RI       A

Human 101 QAECLRYKLLGGLAVRRACYGVLRFIMESGAKGCEVVVSGKLRGQRAKSM  150
Rat
Dros        S    T           Y

Human 151 KFVDGLMIHSGDPVNYYVDTAVRHVLLRQGVLGIKVKIMLPWDPTGKIGP  200
Rat                                                    S
Dros                  C D  E  T                 V   Y  KN

Human 201 KKPLPDHVSIVEPKDEILPTTPISEQKGGKPELPAMPQPVPTA       243
Rat                                    P
Dros           N  V    E KIYE  ET Y IPP SK LDDLSEAKVL       246
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Figure 1. Comparison of the *Drosophila* ribosomal protein S3 to the human and rat ribosomal S3 sequences. Differences of the *Drosophila* and rat sequences are shown below the human sequence.

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